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Partners in Imprinting: Noncoding RNA and Polycomb Group Proteins

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Establishment of genomic imprints during early development involves concerted epigenetic mechanisms. Two recent studies by Terranova et al. (in this issue of *Developmental Cell*) and Pandey et al. (in a recent issue of *Molecular Cell*) have demonstrated that Polycomb group proteins (PcG) and the Kcnq1ot1 regulatory RNA, respectively, are indispensable for gene- and lineage-specific chromatin modification and compaction of the paternally imprinted Kcnq1 cluster.

An intricate parent-of-origin imprinting system has evolved in mammals to silence either a paternal or maternal allele, depending on the locus, while allowing expression of the other allele. Imprinting is critical for dozens of genes important for growth and development. The regulation of imprinted loci has long been studied and is orchestrated by epigenetic factors including regulatory noncoding (ncRNAs), DNA methylation of imprinting control regions (ICRs), posttranslational modifications (PTMs) of histone proteins, and changes in higher-order chromatin structure (Ideraabdullah et al., 2008). However, the underlying mechanistic contributions made by individual components of the epigenetic machinery, and how they cooperate with one another, is still unclear. Pandey et al. (2008) and Terranova et al. (2008) have shed new light on the exquisite regulation of the Kcnq1 imprinted domain.

The Kcnq1 cluster on human chromosome 11p15 spans a 1 Mb region containing approximately 10 paternally imprinted genes, whose expression is regulated by the ncRNA Kcnq1ot1 (Figure 1A). Kcnq1ot1 is transcribed in an antisense fashion from an intron of the Kcnq1 gene and is essential for imprinting; Kcnq1ot1 deletion or promoter ablation results in derepression of paternal imprints (Fitzpatrick et al., 2002; Mancini-Dinardo et al., 2006). However, the basic details of this RNA and its mode of action in establishing lineage-specific monoallelic silencing were not understood. Pandey et al. (2008) have performed an in-depth characterization of Kcnq1ot1 and found that it is 91.5 kb in length (previously described as >60 kb), transcribed by RNA polymerase II (RNAP), retained in the nucleus, and is likely unspliced. While Kcnq1ot1 is critical for imprinting, the key question of whether the act of transcription or the presence of the RNA itself is essential for bidirectional silencing of the imprinted domain remained unanswered. Using an episomal-based system in which the stability of Kcnq1ot1 was regulated by the 3′UTR of a highly unstable transcript, Pandey et al. (2008) demonstrated that Kcnq1ot1 per se is the critical factor. Thus, Kcnq1ot1 officially joins the class of regulatory ncRNAs that includes Xist, which coats the inactive X chromosome (Xi), and Air, encoded by the Igf2r imprinted cluster (Heard and Disteche, 2006; Ideraabdullah et al., 2008).

In order to decipher how Kcnq1ot1 mediates silencing of the Kcnq1 cluster, the authors turned their attention to chromatin. Most genes in the cluster are paternally imprinted in placenta, while a select few near the Kcnq1ot1 transcriptional start site are imprinted in embryonic tissues (nearby or inner genes; Figure 1A). Kcnq1ot1 has been implicated in imprinting both inner and outer genes (Mancini-Dinardo et al., 2006); however, the mechanism by which lineage-specificity is brought about had not been fully explored. Pandey et al. (2008) showed that Kcnq1ot1 interacts with chromatin, but this interaction was several-fold higher in placenta than embryonic liver. To further address the lineage-specific role of Kcnq1ot1 in chromatin regulation, ChIP-chip studies were performed across the Kcnq1 domain using transcriptionally repressive PTMs: H3K27me3 and H3K9me3, as well as transcriptionally active PTMs: H3K4me2 and H3K9ac. H3K27me3 and H3K9me3 were enriched at imprinted genes in placenta and liver, with many regions showing significant enrichment in placenta over liver; H3K4me2 and H3K9ac did not show tissue specific differences. The enrichment of repressive marks has been reported along the Kcnq1 domain (Umlauf et al., 2004), and Pandey et al. (2008) demonstrate that in the case of H3K27me3, Kcnq1ot1 deletion results in the loss of placenta-specific enriched regions. Could it be that Kcnq1ot1 directs histone methyltrasferase (HMTs) complexes to this domain? Indeed, the authors found that in placenta, but not liver, the PcG protein Ezh2 (H3K27 HMT) and G9a (H3K9 HMT) interact with Kcnq1ot1, corresponding to the lineage-specific PTMs. This is consistent with the observation that mice deficient for Eed (required for Ezh2's HMT activity) or G9a have imprinting defects (Mager et al., 2003; Wagschal et al., 2008). Such interactions are exciting in light of recent findings that the ncRNA, HOTAIR, derived from the human HOXC locus, also interacts with EZH2 (Rinn et al., 2007). Combined, these results suggest a possible RNA-directed specificity mechanism for targeting HMT complexes. However, one outstanding question remains—how are these very same repressive PTMs targeted in embryonic liver where Kcnqot1 does not interact with Ezh2 or G9a?

The remarkable differential gene regulation within the Kcnq1 imprinted cluster also prompted Terranova et al. (2008) to study this domain. Here, maternal and paternal allelic differences were examined in preimplantation embryos and extraembryonic trophectodermal stem cells. Using RNA-FISH and confocal microscopy 3D reconstructions, Terranova et al. (2008)

investigated the higher-order chromatin structure of the Kcnq1 cluster. Kcnq1ot1, expressed from the paternal allele, was found to exist in a discrete nuclear compartment that is chock-full of repressive histone PTMs (H3K27me3 and H3K9me3) and PcG proteins such as Ezh2 and Rnf2 (E3-ubiquitin ligase for H2AK119) and generally devoid of H3K4me2 and RNAP (Figure 1B). The maternal allele displayed an opposite pattern, suggesting that the parental clusters exist in different chromatin configurations. Allelic ChIP assays confirmed these results and demonstrated the enrichment of H2AK119u1 at Kcnq1 imprinted genes for the first time.

Terranova et al. (2008) next examined the effects on the Kcnq1 repressive compartment in the absence of Ezh2. Intriguingly, this unique compartment was still intact and devoid of RNAP. So then, what is the function of Ezh2 in regulating the Kcnq1 cluster? The authors found that Ezh2 is required for Kcnq1ot1 association along the length of the cluster, suggesting that Ezh2 may mediate spreading of Kcnq1ot1 and/or play a role in higher-order genomic organization. The latter possibility was explored through measurements of the physical distances between RNA/DNA FISH signals. A paternally contracted genomic state was observed in trophectoderm cells from both WT and Ezh2-deficient blastocysts. However, when extraembryonic ectodermal cells were isolated from Ezh2-deficient postimplantation embryos, the genomic contraction was alleviated. Therefore, Ezh2 maintains the paternally contracted state in vivo; similar results were demonstrated independently for Rnf2. By ChIP analysis, Terranova et al. (2008) showed that in the absence of Ezh2 and Rnf2, paternally imprinted genes along the length of the cluster were derepressed.

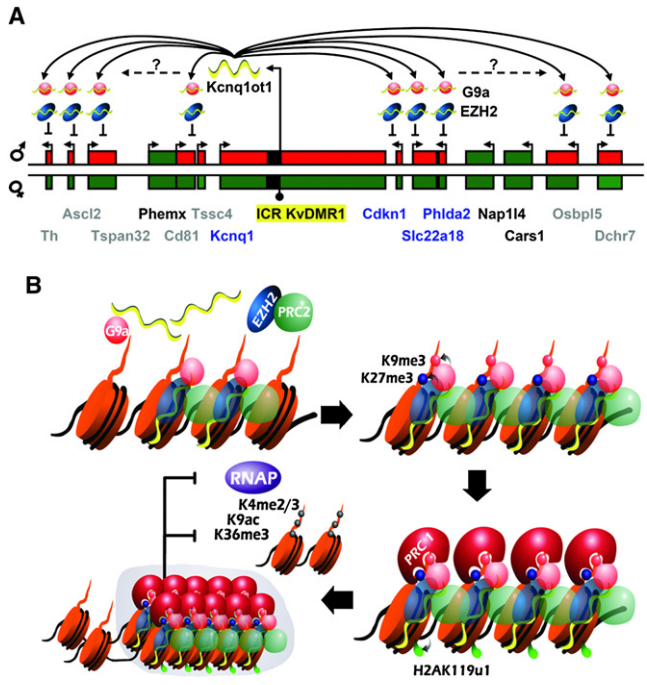

Figure 1. ncRNA and PcG-Mediated Chromatin Compaction of the Kcnq1 Cluster

(A) Kcnq1ot1 transcript regulates Kcnq1 imprinting cluster. Kcnq1ot1-dependent imprinting requires HMTs G9a and Ezh2 and their interaction with Kcnq1ot1 RNA appears critical for paternal imprinting in extraembryonic tissue. The dashed line represents the possible mechanism of spreading repression by HMT complexes containing Kcnq1ot1. Genes in blue text indicate "inner" imprinted genes, in gray text indicate "outer" imprinted genes, and in black text indicate nonimprinted genes. Paternal and maternal alleles are indicated. Red boxes represent paternally imprinted genes, and green boxes represent paternally and maternally expressed genes. The black circle represents DNA methylation of Kcnq1ot1 promoter (KvDMR1) on maternal allele.

(B) Polycomb repressive complex 2 (PRC2) containing Ezh2 and G9a associate with Kcnq1ot1 and are recruited to imprinted genes in Kcnq1 cluster. Repressive histone PTMs H3K9me3 and H3K27me3 are directed by G9a and Ezh2, respectively. Small pink circles on histone tails indicate H3K9me3; small blue circles represent H3K27me3. PRC1 complex recognizes repressive histone PTMs via PcG protein interaction, and PRC1 member Rnf2 catalyzes the repressive PTM H2AK119u1. However, PRC1 targeting may be independent of PRC2 as shown by Terranova et al. (2008). Ubiquitin is depicted with green circles. PRC1 and PRC2 complexes direct genomic contraction and higher-order chromatin condensation. Shaded area represents a distinct nuclear repressive compartment, devoid of RNAP and active histone PTMs, H3K4me3, H3K9ac and H3K36me3, indicated by small gray circles.

The mechanistic details and kinetics of the Kcnq1 imprinted domain are reminiscent of the Xi. In both cases, an ncRNA is essential for the initiation of silencing, multiple stepwise events are involved in establishing repression (including PcG recruitment), and silencing ensues through the nucleation of a repressive compartment devoid of transcription factors (Chaumeil et al., 2006). This raises an interesting paradox regarding transcription of Kcnq1ot1 itself, in which Terranova et al. (2008) observe partial focal colocalization with RNAP. Furthermore, how are nonimprinted genes protected from the action of repressive HMTs (shown by Pandey et al. [2008])? How are nonimprinted genes organized within the repressive compartment? One major difference between Kcnq1 and Xi is that only Kcnq1ot1 interacts with HMTs, suggesting potential mechanistic differences in HMT targeting at these loci. While questions still abound, these Kcnq1 studies have brought us few steps closer to understanding the intricate molecular mechanisms of imprinting.

REFERENCES

Chaumeil, J., Le Baccon, P., Wutz, A., and Heard, E. (2006). Genes Dev. *20*, 2223–2237.

Fitzpatrick, G.V., Soloway, P.D., and Higgins, M.J. (2002). Nat. Genet. *9*, 426–431.

Heard, E., and Disteche, C.M. (2006). Genes Dev. *20*, 1848–1867.

Ideraabdullah, F.Y., Vigneau, S., and Bartolomei, M.S. (2008). Mutat. Res. Published online August 20, 2008. 10.1016/j.mrfmmm.2008.08.008.

Mager, J., Montgomery, N.D., de Villena, F.P., and Magnuson, T. (2003). Nat. Genet. *33*, 502–507.

Mancini-Dinardo, D., Steele, S.J., Levorse, J.M., Ingram, R.S., and Tilghman, S.M. (2006). Genes Dev. *20*, 1268–1282.

Pandey, R.R., Mondal, T., Mohammad, F., Enroth, S., Redrup, L., Komorowski, J., Mancini- DiNardo, D., and Kanduri, C. (2008). Mol. Cell *32*, 232–246.

Rinn, J.L., Kertesz, M., Wang, J.K., Squazzo, S.L., Xu, X., Brugmann, S.A., Goodnough, L.H., Helms, J.A., Farnham, P.J., Segal, E., and Chang, H.Y. (2007). Cell *129*, 1311–1323.

Terranova, R., Yokobayashi, S., Stadler, M.B., Otte, A.P., van Lohuizen, M., Orkin, S.H., and Peters, A.H.F.M. (2008). Dev. Cell *15*, this issue, 668–679.

Umlauf, D., Goto, Y., Cao, R., Cerqueira, F., Wagschal, A., Zhang, Y., and Feil, R. (2004). Nat. Genet. *36*, 1296–1300.

Wagschal, A., Sutherland, H.G., Woodfine, K., Henckel, A., Chebli, K., Schulz, R., Oakey, R.J., Bickmore, W.A., and Feil, R. (2008). Mol. Cell. Biol. *28*, 1104–1113.